Supporting Information

Discovery of a Selective Series of Inhibitors of *Plasmodium falciparum* HDACs.

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General experimental details

Solvents and reagents were obtained from commercial suppliers and were used without further purification. Flash chromatography purifications were performed on prepacked cartridges on a Biotage system. Purity of final compounds were determined using MS and UPLC. UPLC-MS analyses were performed on a Waters Acquity UPLC^{TM}, equipped with a diode array and a ZQ mass spectrometer, using an X-Terra C18 column (5 μm, 4.6 x 50 mm) or a BEH C18 column (1.7 μm, 2.1 x 50 mm). Mobile phase comprised a linear gradient of binary mixtures of H_2O containing 0.1% formic acid (A), and MeCN containing 0.1% formic acid (B). The linear gradient used is: (A): 90% (0.1 min), 90%-0% (2.6 min), 0% (0.3 min), 0%-90% (0.1 min) with a 0.5 mL/min flow.

Purity of final compounds were ≥95%. High-resolution mass spectra were recorded on a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific) operating in positive direct infusion. The resolution used was 140000 full width half medium (at 200 m/z). Maximum tolerance for the accuracy was set to 5 ppm. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV400 and AV600 spectrometers. NMR spectra were recorded at 400 or 600 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. ^{13}C NMR signals were assigned through ^{1}H-^{13}C HSQC and HMBC spectra. Chemical shift (δ) are reported in parts per million relative to TMS using $CDCl_3$ as a solvent or proteo impurity of the solvent using DMSO-d_6. Coupling costants (*J*) are reported in Hertz (Hz). Multiplicities are reported as singlet (s), broad (br), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), triplet (t), doublet of triplet (dt) or multiplet (m). Unless indicated, spectra were acquired at 300 K. Temperatures are expressed in degrees Celsius (°C) and are uncorrected. The authors thanks Dr. Mariana Gallo for the acquisition and assignation of ^{13}C NMR spectra.

Abbreviations

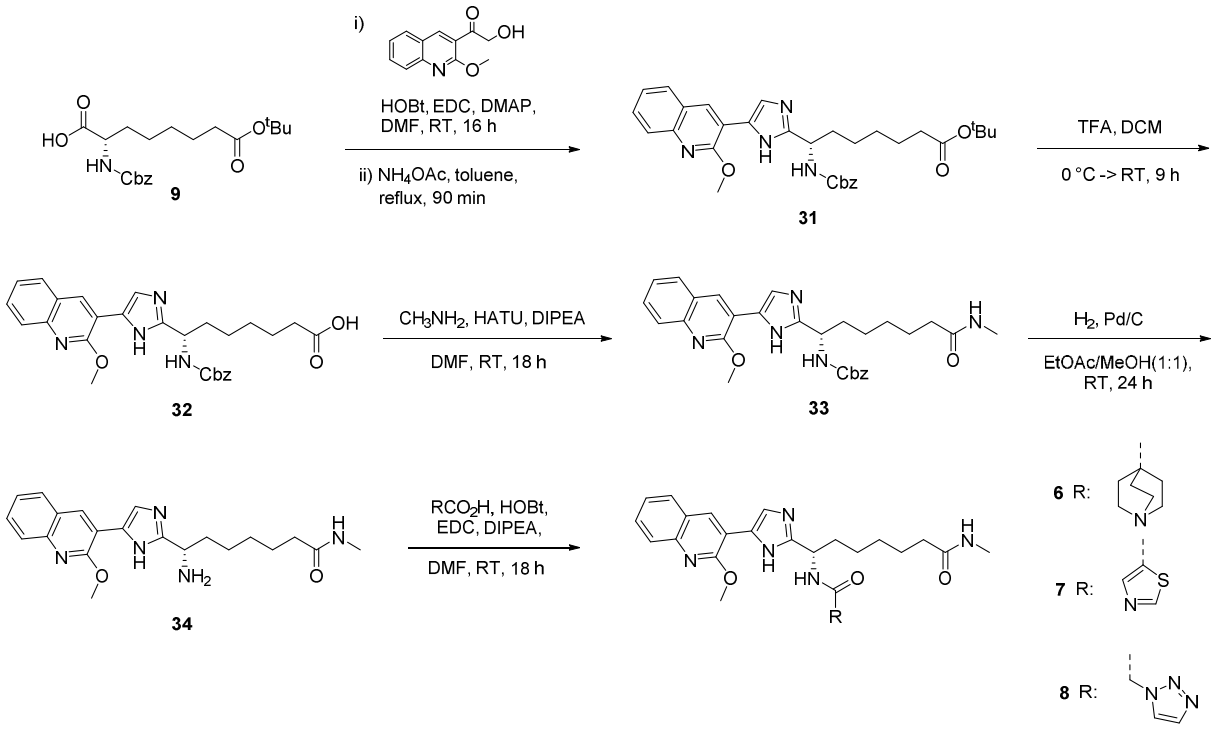
AcOH:	Acetic acid
DCM:	Dichloromethane
DIPEA:	*N*,*N*-Diisopropylethylamine
DMAP:	4-(Dimethylamino)pyridine
DME:	1,2-Dimethoxyethane
DMF:	Dimethylformamide
DMSO:	Dimethylsulfoxide
EDC·HCl:	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
Eq:	equivalent
ES^{+}:	Electrospray Positive Ionisation
EtOAc:	Ethyl acetate
EtOH:	Ethanol
Et_2O:	Diethyl ether
h:	hour
HATU:	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBTU:	*N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate, *O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate
hERG:	Human Ether-a-go-go-Related Gene
HOBt:	1-Hydroxybenzotriazole hydrate

HPLC:	High Performance Liquid Chromatography
HUVEC:	Human Umbilical Vein Endothelial Cell
LCMS:	Liquid Chromatography Mass Spectrometry
MeCN:	Acetonitrile
MeOH:	Methanol
min:	minute
NIS:	*N*-Iodosuccinimide
$PdCl_2$(dppf)	[1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium (II)
RP:	Reverse Phase
RT:	Retention time
TBTU:	*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate
TFA:	Trifluoroacetic acid
THF:	Tetrahydrofurane

Synthetic experimental details for compounds 6-8, 10-13 and 15-30[a]

[a]Compounds **4, 5, 9** and **14** were synthesized using the procedures described in: Atenni, B.; Ferrigno, F.; Jones, P.; Ingenito, R.; Kinzel, O.; Llauger-Bufi, L.; Ontoria, J. M.; Pescatore, G.; Rowley, M.; Scarpelli, R.; Schultz-Fademrecht, C. Heterocycles Derivatives as Histone Deacetylase (HDAC) Inhibitors. WIPO Patent 061638, 2006.

Scheme 1: Synthesis of compounds 6-8



***tert*-Butyl (*S*)-7-(((benzyloxy)carbonyl)amino)-7-(5-(2-methoxyquinolin-3-yl)-1*H*-imidazol-2-yl)heptanoate (31)**

A solution of (*S*)-2-(((benzyloxy)carbonyl)amino)-8-(*tert*-butoxy)-8-oxooctanoic acid **9** (prepared as described in WO2006/061638) (323 mg, 0.85 mmol), *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (230 mg, 1.2 mmol) and HOBt (193 mg, 1.06 mmol) were dissolved in anhydrous DMF (2 mL) and stirred for 15 min. The resulting solution was added to a mixture of 2-hydroxy-1-(2-methoxyquinolin-3-yl)ethanone (189 mg, 0.85 mmol) (prepared as described in *J. Med. Chem.* **2009**, *59*, 3453-3456) and DMAP (32 mg, 0.26 mmol) in anhydrous DMF (0.5 mL). After stirring at room temperature for 16 h the mixture was partitioned between DCM and water. The organic phase was washed with water and brine and dried over Na_2SO_4. Concentration under reduced pressure gave an oily crude (492 mg) which was used as such. A solution of the resulting oil and ammonium acetate (1.31 g, 17 mmol) in toluene (5 mL) was refluxed under Dean-Stark conditions for 1 h 30 min. After cooling to room temperature the mixture was diluted with EtOAc. The organic phase was washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure to give a crude that was purified by column chromatography (eluent: petroleum ether/EtOAc from 7:3 to 2:3) to give the title compound as a yellow powder (274 mg, 58%). ^{1}H-NMR ($CDCl_3$, 400MHz) δ 8.69 (s, 1H), 7.93 (br s, 1H), 7.92 (d, 1H, J = 7.2 Hz), 7.85 (br s, 1H), 7.80 (d, 1H, J = 8.0 Hz), 7.68 (t, 1H, J = 7.6 Hz), 7.47 (t, 1H, J = 7.4 Hz), 7.40-7.25 (m, 4H), 5.04 (dd, 2H, J = 32, 12.4 Hz), 4.82 (m, 1H), 4.12 (s, 3H), 2.15 (t, 2H, J = 7.4 Hz), 1.88 (m, 2H), 1.45 (m, 2H), 1.35 (s, 9H), 1.27 (m, 4H). LCMS (ES^+) m/z 559 $(M+H)^+$.

(*S*)-3-(2-(1-(((Benzyloxy)carbonyl)amino)-6-carboxyhexyl)-1*H*-imidazol-3-ium-5-yl)-2-methoxyquinolin-1-ium bis trifluoroacetate (32)

To a solution of **31** (272 mg, 0.49 mmol) in DCM (4.5 mL) cooled to 0 °C was slowly added TFA (0.5 mL). Reaction mixture was stirred at 0 °C for 1 h, then left warming at room temperature and stirred for 8 h. Volatiles were removed under reduced pressure, the resulting oily residue was co-evaporated first with toluene then with Et_2O to give the title compound (246 mg, quantitative yield) which was used as such in the next step. LCMS (ES^+) m/z 503 $(M+H)^+$.

(*S*)-Benzyl (1-(5-(2-methoxyquinolin-3-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate (33)

To a solution of **32** (180 mg, 0.358 mmol) in DMF (3 mL) were added HATU (272 mg, 0.716 mmol) and DIPEA (0.19 mL, 1.074 mmol) and the resulting solution was stirred for 30 min at room temperature. Then methylamine (2.0 M solution in THF, 0.9 mL, 1.79 mmol) was added. The

reaction mixture was stirred at room temperature for 18 h and diluted with EtOAc, washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude was purified by column chromatography (eluent: DCM/MeOH from 100:0 to 9:1) to get the title compound as a pale yellow solid (140 mg, 76%). LCMS (ES^+) m/z 516 $(M+H)^+$.

(*S*)-7-Amino-7-(5-(2-methoxyquinolin-3-yl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide (34)

Intermediate **33** (140 mg, 0.272 mmol) was dissolved in EtOAc/MeOH (1:1) (30 mL) and treated with Pd/C (14 mg, 10% w/w). The resulting mixture was purged with N_2 and stirred under H_2 atmosphere at atmospheric pressure and room temperature for 24 h. Then, reaction mixture was filtered through a pad of SolKa Floc and filtrate was concentrated under vacuum to give the title compound as pale yellow solid (103 mg, quantitative yield) which was used as such in the next step. LCMS (ES^+) m/z 382 $(M+H)^+$.

General procedure of amide coupling for the synthesis of compounds 6, 7, and 8

To a stirred solution of **34** (1.0 eq.) in DMF (0.14 M) was added a pre-stirred (15 min) solution of carboxylic acid (1.25 eq.), HOBt (1.25 eq.), EDC·HCl (1.25 eq.) and DIPEA (2 eq.) in DMF (0.3 M). The reaction mixture was stirred at room temperature for 18 h and subsequently was purified by RP-HPLC ($MeCN/H_2O$ + 0.1 % TFA). The product was obtained as TFA salt which was partitioned between DCM and sat. aq. $NaHCO_3$. The organic phase was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting syrup was dissolved in $MeCN/H_2O$ (1:1) and treated with L-tartaric acid (1.0 eq. based on TFA salt obtained). The resulting solution was lyophilized to obtain the title compound as mono *L*-tartrate salt.

(*S*)-4-((1-(5-(2-Methoxyquinolin-1-ium-3-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamoyl)quinuclidin-1-ium mono *L*-tartrate salt (6)

Prepared according to general amide coupling procedure using **34** (40 mg, 0.108 mmol), 4-carboxyquinuclidin-1-ium chlorhydrate (22 mg, 0.115 mmol), HOBt (15.5 mg, 0.115 mmol), EDC·HCl (22.1 mg, 0.115 mmol) and DIPEA (44 µL, 0.252 mmol) in DMF (1.5 mL). The title compound was obtained as mono *L*-tartrate salt (24 mg, 49%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.73 (br s, 1H), 7.97 (d, 1H, *J* = 8.4 Hz), 7.92 (d, 1H, *J* = 7.6 Hz), 7.76 (d, 1H, *J* = 8.4 Hz), 7.66 (m, 1H), 7.61 (m, 2H), 7.42 (t, 1H, *J* = 7.4 Hz), 5.02 (m, 1H), 4.13 (s, 3H), 3.99 (s, 2H, CH tartrate), 3.21 (t, 6H, *J* = 7.8 Hz), 2.54 (d, 3H, *J* = 4.4 Hz), 2.03 (t, 2H, *J* = 7.4 Hz), 1.96 (t, 6H, *J* = 8.0 Hz), 1.47 (m, 2H), 1.26 (m, 6H). LCMS (ES^+) m/z 519 $(M+H)^+$. HPLC purity: > 98%, RT: 0.94 min. ^{13}C-NMR (DMSO-d_6, 150 MHz) δ 174.3, 173.8, 158.2, 149.3, 145.0, 135.4, 130.6, 128.3,

126.9, 125.4, 124.6, 119.3, 54.3, 47.2, 45.9, 42.4, 35.7, 32.9, 28.5, 25.6, 25.5, 25.3. HRMS calcd for $[C_{29}H_{39}N_6O_3 + H]^+$: 519.3078, found: 519.3075.

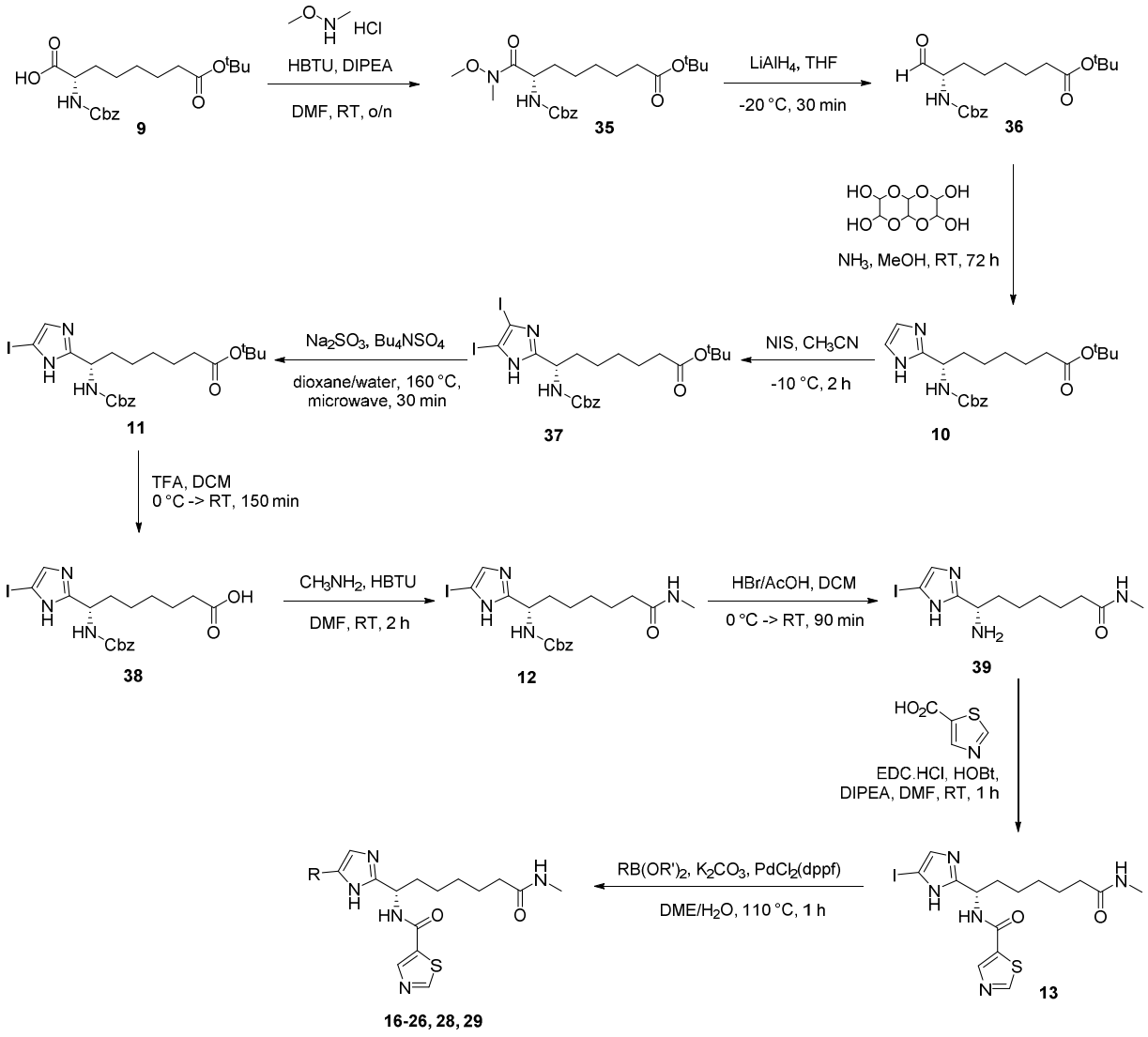
<u>*N*-(1-(5-(2-Methoxyquinolin-3-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide mono *L*-tartrate salt (7)</u>

Prepared according to general procedure of amide coupling using **34** (34 mg, 0.089 mmol), thiazole-5-carboxylic acid (15 mg, 0.116 mmol), HOBt (15.7 mg, 0.116 mmol), EDC·HCl (22.2 mg, 0.116 mmol) and DIPEA (23.3 µL, 0.134 mmol) in DMF (1.0 mL). The title compound was obtained as mono *L*-tartrate salt (23.5 mg, 41%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.23 (s, 1H), 9.05 (d, 1H, *J* = 8.4 Hz), 8.73 (br, 1H), 8.61 (s, 1H), 8.06 (s, 2H), 7.93 (d, 1H, *J* = 7.6 Hz), 7.75 (d, 1H, *J* = 8.4 Hz), 7.68-7.55 (m, 3H), 7.41 (t, 1H, *J* = 7.4 Hz), 5.15 (m, 1H), 4.28 (s, 3H), 4.12 (s, 2H, CH tartrate), 2.54 (d, 3H, *J* = 4.8 Hz), 2.10 (m, 1H), 2.03 (t, 2H, *J* = 7.4 Hz), 1.50 (m, 2H), 1.45-1.20 (m, 4H). LCMS (ES^+) m/z 493 $(M+H)^+$. HPLC purity: > 95%, RT: 1.18 min. ^{13}C-NMR (DMSO-d_6, 150 MHz) δ 158.3, 144.3, 133.7, 133.0, 129.0, 128.0, 126.7, 125.7, 124.7, 118.0, 54.0, 48.1, 35.8, 33.5, 28.8, 25.9, 25.6. HRMS calcd for $[C_{25}H_{29}N_6O_3S + H]^+$: 493.2016, found: 493.2010.

<u>7-(2-(1*H*-1,2,3-Triazol-1-yl)acetamido)-7-(5-(2-methoxyquinolin-3-yl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide mono *L*-tartrate salt (8)</u>

Prepared according to general amide coupling procedure using **34** (34 mg, 0.089 mmol), 2-(1*H*-1,2,3-triazol-1-yl)acetic acid (14.7 mg, 0.116 mmol), HOBt (15.7 mg, 0.116 mmol), EDC·HCl (22.2 mg, 0.116 mmol) and DIPEA (23.3 µL, 0.1337 mmol) in DMF (1.0 mL). The title compound was obtained as mono *L*-tartrate salt (28.5 mg, 50%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.76 (br s, 1H), 8.69 (d, 1H, *J* = 8.0 Hz), 7.95 (d, 1H, *J* = 8.0 Hz), 7.80 (s, 1H), 7.76 (d, 1H, *J* = 8.0 Hz), 7.62 (m, 2H), 7.42 (t, 1H, *J* = 7.6 Hz), 5.22 (s, 2H), 4.95 (m, 1H), 4.22 (s, 2H, CH tartrate), 4.13 (s, 3H), 2.54 (d, 3H, *J* = 4.8 Hz), 2.03 (t, 2H, *J* = 7.4 Hz), 1.97 (m, 1H), 1.81 (m, 1H), 1.47 (m, 2H), 1.27 (m, 4H). LCMS (ES^+) m/z 491 $(M+H)^+$. HPLC purity: > 95%, RT: 1.16 min. ^{13}C-NMR (DMSO-d_6, 150 MHz) δ 158.5, 145.4, 135.9, 135.4, 130.7, 128.4, 127.0, 125.4, 119.3, 56.5, 53.9, 48.1, 35.8, 33.8, 28.8, 26.8, 25.7. HRMS calcd for $[C_{25}H_{31}N_8O_3 + H]^+$: 491.2514, found: 491.2508.

Scheme 2: Synthesis of compounds 16-26, 28, 29



(*S*)-*tert*-Butyl 7-(((benzyloxy)carbonyl)amino)-8-(methoxy(methyl)amino)-8-oxooctanoate (35)

A solution of (*S*)-2-(((benzyloxy)carbonyl)amino)-8-(*tert*-butoxy)-8-oxooctanoic acid **9** (6.6 g, 17.39 mmol), HBTU (7.25 g, 19.13 mmol) and *N*,*N*-diisopropylethylamine (6.06 mL, 34.78 mmol), were stirred in DMF (25 mL) for 10 min at room temperature before dimethylhydroxylamine hydrochloride (1.87 g, 19.13 mmol) was added. The reaction mixture was stirred for a further 18 h at room temperature then diluted with EtOAc. Organic phase was washed with sat. aq. $NaHCO_3$, sat. aq. NaCl, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oily residue was purified by column chromatography (eluent: Petroleum ether/EtOAc from 85/15 to 1/4 v/v) affording the desired product **35** as pale yellow oil (6.87 g, 93%). ^{1}H-NMR ($CDCl_3$, 400MHz) δ 7.40-7.27 (m, 5H), 5.41 (d, 1H, *J* = 8.8 Hz), 5.90 (dd, 2H, *J* = 12.2, 22.6 Hz), 4.72 (m, 1H), 3.77

(s, 3H), 3.20 (s, 3H), 2.18 (t, 2H, J = 7.4 Hz), 1.80-1.20 (m, 10H), 1.43 (s, 9H). LCMS (ES^+) m/z 423 $(M+H)^+$. HPLC purity: > 90%, RT: 2.13 min.

(*S*)-*tert*-Butyl 7-(((benzyloxy)carbonyl)amino)-8-oxooctanoate (36)

To a stirred solution of the Weinreb amide **35** (2.93 g, 6.93 mmol) in THF (69 mL) cooled to -20 °C, was added dropwise a 1.0 M solution of $LiAlH_4$ in THF (9.0 mL, 9.00 mmol). Reaction mixture was stirred for 30 min at -20 °C before quenching with EtOAc. Reaction mixture was subsequently partitioned between EtOAc and 0.1 M aq. HCl sol. The organic phase was separated and the aqueous phase extracted into EtOAc. The combined organic extracts were washed with H_2O, sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo* to give the desired product **36** as pale yellow oil (2.52 g, quantitative yield) which was used in the next step without further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.59 (s, 1H), 7.40-7.27 (m, 5H), 5.30 (m, 1H), 5.12 (s, 2H), 4.31 (m, 1H), 2.19 (t, 2H, J = 7.4 Hz), 2.00-1.85 (m, 1H), 1.70-1.20 (m, 9H), 1.43 (s, 9H). LCMS (ES^+) m/z 364 $(M+H)^+$.

(*S*)-*tert*-Butyl 7-(((benzyloxy)carbonyl)amino)-7-(1*H*-imidazol-2-yl)heptanoate (10)

To a stirred solution of the aldehyde **36** (2.52 g, 6.93 mmol) in MeOH (17 mL) at room temperature was added glyoxal trimer dihydrated (1.46 g, 6.93 mmol) followed by slowly addition of ammonia (7.0 M in MeOH, 5.0 mL, 34.67 mmol). Reaction mixture was stirred in a sealed tube at room temperature for 72 h performing 3 subsequent additions of both glyoxal trimer dihydrated and ammonia until no more progress of reaction was observed. Volatiles were removed under reduced pressure and residue was purified by column chromatography (eluent: Petroleum ether/EtOAc from 4/1 to 0/10 v/v) affording the desired product **10** as yellow solid (2.37 g, 85%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.90 (br s, 1H), 7.39-7.28 (m, 5H), 6.96 (s, 2H), 5.34 (d, 1H, J = 7.2 Hz), 5.09 (dd, 2H, J =12.4, 20.4 Hz), 4.69 (m, 1H), 2.18 (t, 2H, J = 7.4 Hz), 1.99-1.84 (m, 1H), 1.70-1.30 (m, 9H), 1.43 (s, 9H). LCMS (ES^+) m/z 402 $(M+H)^+$.

(*S*)-*tert*-Butyl 7-(((benzyloxy)carbonyl)amino)-7-(4,5-diiodo-1*H*-imidazol-2-yl)heptanoate (37)

To a stirred solution of imidazole **10** (1.0 g, 2.49 mmol) in MeCN (50 mL) cooled to -10 °C was added portionwise NIS (1.68 g, 7.47 mmol). Reaction mixture was stirred at -10 °C for 1 h then 2 subsequent additions of NIS (0.56 g, 2.49 mmol) after 30 min each were performed until completion of reaction was observed. Reaction mixture was allowed to warm up to room temperature then MeCN was removed under reduced pressure. Residue was dissolved with EtOAc and washed with sat. aq. $NaHCO_3$, sat. aq. $Na_2S_2O_3$, sat. aq. NaCl, dried over Na_2SO_4 and

concentrated *in vacuo* to give the desired product **37** as brownie oily residue which was used in the next step without further purification (1.63 g, quantitative yield). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.11 (br s, 1H), 7.40-7.27 (m, 5H), 5.90 (m, 1H), 5.10 (m, 2H), 4.70 (m, 1H), 2.20 (t, 2H, *J* = 7.2 Hz), 2.10-1.95 (m, 1H), 1.80-1.20 (m, 9H), 1.44 (s, 9H). LCMS (ES^+) m/z 654 $(M+H)^+$.

(*S*)-*tert*-Butyl 7-(((benzyloxy)carbonyl)amino)-7-(5-iodo-1*H*-imidazol-2-yl)heptanoate (11)

To a stirred solution of diiodo derivative **37** (1.63 g, 2.49 mmol) in 1,4-dioxane/H_2O solution (4/1 v/v, 25 mL) were added sodium sulfite (3.14 g, 24.91 mmol) and tetrabuthylammonium hydrogen phosphate (1.69 g, 4.98 mmol). Reaction mixture was heated in the microwave at 160 °C for 30 min then 1,4-dioxane was removed under reduced pressure and aqueous phase was extracted with EtOAc. Organic phase was washed with sat. aq. $NaHCO_3$, sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was purified by column chromatography (eluent: Petroleum ether/EtOAc from 4/1 to 1/4 v/v) affording the desired product **11** as a yellow oil (0.88 g, 67%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.40-7.24 (m, 5H), 7.02 (s, 1H), 6.01 (m, 1H), 5.06 (dd, 2H, *J* = 12.6, 26.6 Hz), 4.69 (m, 1H), 2.18 (t, 2H, *J* = 7.2 Hz), 2.13-2.00 (m, 1H), 2.00-1.86 (m, 1H), 1.60-1.48 (m, 2H), 1.43 (s, 9H), 1.40-1.22 (m, 4H). LCMS (ES^+) m/z 528 $(M+H)^+$. HPLC purity: > 90%, RT: 1.83 min.

(*S*)-7-(((Benzyloxy)carbonyl)amino)-7-(5-iodo-1*H*-imidazol-2-yl)heptanoic acid (38)

Prepared as described for compound **32** using the iodo derivative **11** (4.93 g, 9.35 mmol) in DCM (50 mL)/TFA (25 mL) and stirred for 30 min at 0 °C then at room temperature for 2 h. The desired product **38** was obtained as pale brown sticky solid (5.24 g, 96%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.93 (d, 1H, *J* = 6.8 Hz), 7.58 (s, 1H), 7.44-7.28 (m, 5H), 5.04 (dd, 2H, *J* = 12.6, 32.2 Hz), 4.72 (m, 1H, CH), 2.18 (t, 2H, *J* = 7.2 Hz), 1.90-1.70 (m, 2H), 1.53-1.40 (m, 2H), 1.35-1.15 (m, 4H). LCMS (ES^+) m/z 472 $(M+H)^+$. HPLC purity: > 90%, RT: 1.25 min.

(*S*)-Benzyl (1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate (12)

To a stirred solution of carboxylic acid **38** (6.9 g, 11.79 mmol) in DMF (39 mL) was added methylamine (2.0 M solution in THF, 30 mL, 60 mmol) followed by HBTU (8.94 g, 23.57 mmol). Solution pH was adjusted to basic value by addition of methylamine (2.0 M solution in THF, 15 mL, 30 mmol). Reaction mixture was stirred at room temperature for 1 h then two more addition of HBTU (4.47 g, 28.5 mmol) and methylamine (2.0 M solution in THF, 15 mL, 30 mmol) every 30 min were performed to reach completion of reaction. THF was evaporated *in vacuo* and DMF residue was diluted with EtOAc, washed with sat. aq. $NaHCO_3$, sat. aq. NaCl, dried over Na_2SO_4

and concentrated *in vacuo*. The resulting oily residue was purified by column chromatography (eluent: Petroleum ether/EtOAc from 4/1 to 0/10 v/v) affording the desired product **12** as white sticky solid (5.48 g, 96%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 12.11 (br s, 1H), 7.69-7.59 (m, 2H), 7.41-7.27 (m, 4H), 7.19 (s, 1H), 5.03 (dd, 2H, , *J* = 12.8, 22.8 Hz), 4.57 (m, 1H), 2.54 (d, 3H, *J* = 4.8 Hz), 2.01 (t, 2H, *J* = 7.4 Hz), 1.85-1.60 (m, 2H), 1.50-1.38 (m, 2H), 1.32-1.12 (m, 4H). LCMS (ES^+) m/z 485 $(M+H)^+$. HPLC purity: > 90%, RT: 1.16 min.

(*S*)-7-Amino-7-(5-iodo-1*H*-imidazol-2-yl)-*N*-methylheptanamide (39)

A solution of (*S*)-benzyl (1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate **12** (1.5 g, 3.097 mmol) in DCM (40 mL) was treated with a solution of HBr in AcOH (20 mL) at 0°C and the reaction mixture was stirred at this temperature for 1 h then allowed to warm up to room temperature and stirred for 30 min. Solvents were removed *in vacuo* and excess reagents were removed by repeated addition of toluene and evaporation of the solvent. The residue obtained was dissolved in MeOH, passed through a SCX resin (20 g) and washed with MeOH. After treatment of the resin with NH_3 in MeOH (7 N) and removal of solvent *in vacuo* the desired product **39** was afforded as a pale orange powder (1.08 g, quantitative yield). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.67 (br s, 1H), 7.13 (s, 1H), 3.74 (m, 1H), 2.54 (d, 3H, *J* = 4.8 Hz), 2.01 (m, 2H), 1.64 (m, 1H), 1.50 (m, 3H), 1.22 (m, 4H). LCMS (ES^+) m/z 351 $(M+H)^+$. HPLC purity: > 90%, RT: 0.63 min.

***(S)*-*N*-(1-(5-Iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (13)**

Prepared according to general amide coupling procedure using (*S*)-7-amino-7-(5-iodo-1*H*-imidazol-2-yl)-*N*-methylheptanamide **39** (1.08 g, 3.097 mmol), thiazole-5-carboxylic acid (0.6 g, 4.6455 mmol), EDC·HCl (0.89 g, 4.6455 mmol), HOBt (0.63 g, 4.6455 mmol) and DIPEA (1.2 mL, 6.89 mmol) in DMF (15 mL). The reaction was stirred for 1 h at room temperature and subsequently partitioned between DCM and sat. aq. $NaHCO_3$. The organic phase was separated and the aqueous phase extracted with DCM. The combined organic extracts were washed with sat. aq. NaCl, dried over Na_2SO_4 and concentrated *in vacuo* to give a residue which was purified by flash chromatography (eluent: DCM/MeOH from 100/0 to 85/15 v/v) to afford the desired product **13** as a white powder (1.13 g, 79%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 12.25 (br s, 1H), 9.23 (s, 1H), 9.02 (d, 1H, *J* = 8.0 Hz), 8.58 (s, 1H), 7.65 (br s, 1H), 7.23 (s, 1H), 5.05 (m, 1H), 2.55 (d, 3H, *J* = 5.2 Hz), 2.02 (t, 2H, *J* = 7.6 Hz), 1.97 (m, 1H), 1.82 (m, 1H), 1.47 (m, 2H), 1.25 (m, 4H). LCMS (ES^+) m/z 462 $(M+H)^+$.

General Suzuki coupling procedure for the synthesis of compounds 16-26, 28, 29

A degassed microwave vial was charged with (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (1.0 eq.), boronic acid or ester (2.0 eq.), $PdCl_2$(dppf) (0.2 eq.) and potassium carbonate (3.0 eq.). A 1/1 degassed solution of DME/H_2O (0.05 M) was added and the suspension was degassed for further 10 min and then heated at 110 °C for 1 h. After cooling, solvents were removed *in vacuo* and residue dissolved in DMF and filtered. The crude material was purified by preparative RP-HPLC to get after lyophilization the title products **16-26, 28, 29**.

***N*-(7-(Methylamino)-7-oxo-1-(5-(quinolin-8-yl)-1*H*-imidazol-2-yl)heptyl)thiazole-5-carboxamide (16)**

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (20 mg, 0.043 mmol), quinolin-8-ylboronic acid (15 mg, 0.086 mmol), $PdCl_2$(dppf) (6.1 mg, 0.0086 mmol) and potassium carbonate (18 mg, 0.13 mmol) in 1/1 degassed solution of DME/H_2O (0.9 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. To drive the reaction to completion quinolin-8-ylboronic acid (15 mg, 0.086 mmol), $PdCl_2$(dppf) (6.1 mg, 0.0086 mmol) and degassed DME (0.5 mL) were added again. Reaction mixture was degassed for further 5 min and heated for 1 h. then cooled to room temperature. The title compound was obtained as pale yellow solid (3.2 mg, 11%). ^{1}H-NMR (DMSO-d_6, 600 MHz) δ 9.26 (s, 1H), 9.05 (dd, 2H, *J* = 4.2, 1.8 Hz), 8.64 (s, 1H), 8.54 (dd, 1H, *J* = 8.4, 1.7 Hz), 8.31 (s, 1H), 8.22 (d, 1H, *J* = 7.2 Hz), 8.12 (d, 1H, *J* = 8.0 Hz), 7.77 (t, 2H, *J* = 7.7 Hz), 7.71 (dd, 2H, *J* = 8.3, 4.2 Hz), 7.67 (br m, 1H), 5.37 (t, 2H, *J* = 7.5 Hz), 2.60-2.50 (m, 3H), 2.09-2.06 (m, 2H), 2.05-2.02 (m, 2H), 1.54-1.48 (m, 2H), 1.46-1.42 (m, 2H), 1.38-1.28 (m, 2H). LCMS (ES^+) m/z 463 $(M+H)^+$. HPLC purity: > 99%, RT: 1.02 min.

***N*-(1-(5-(1*H*-Indol-5-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (17)**

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (31 mg, 0.0672 mmol), (1*H*-indol-5-yl)boronic acid (21.6 mg, 0.134 mmol), $PdCl_2$(dppf) (9.4 mg, 0.0134 mmol) and potassium carbonate (28 mg, 0.201 mmol) in 1/1 degassed solution of DME/H_2O (1.3 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound was obtained as white solid (15.2 mg, 40%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 14.35 (br s, 1H), 11.34 (s, 1H), 9.24 (d, 1H, *J* = 6.4 Hz), 8.63 (s, 1H), 7.99 (s, 1H), 7.93 (br s, 1H), 7.66 (m, 1H), 7.55-7.42 (m, 3H), 6.52

(s, 1H), 5.24 (m, 1H), 2.53 (d, 3H, *J* = 4.4 Hz), 2.15-1.95 (m, 2H), 2.04 (t, 2H, *J* = 7.4 Hz), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 451 $(M+H)^+$. HPLC purity: > 99%, RT: 0.89 min.

<u>*N*-(1-(5-(1*H*-Indazol-5-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (18)</u>

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (30 mg, 0.065 mmol), (1*H*-indazol-5-yl)boronic acid (21.1 mg, 0.13 mmol), $PdCl_2$(dppf) (9.5 mg, 0.013 mmol) and potassium carbonate (27 mg, 0.195 mmol) in 1/1 degassed solution of DME/H_2O (1.2 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound **18** was obtained as white solid (11.2 mg, 30%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.29 (s, 1H), 9.25 (d, 1H, *J* = 6.4 Hz), 8.64 (s, 2H), 8.21 (s, 1H), 8.0 (br s, 1H), 7.80-7.60 (m, 2H), 5.24 (m, 1H), 2.53 (d, 3H, *J* = 4.4 Hz), 2.20-1.95 (m, 2H), 2.04 (t, 2H, *J* = 7.4 Hz), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 452 $(M+H)^+$. HPLC purity: > 95%, RT: 0.77 min.

<u>*N*-(1-(5-(1*H*-Benzo[d]imidazol-5-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (19)</u>

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (50 mg, 0.108 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzo[d]imidazole (53 mg, 0.217 mmol), $PdCl_2$(dppf) (15.2 mg, 0.002 mmol) and potassium carbonate (44.9 mg, 0.325 mmol) in 1/1 degassed solution of DME/H_2O (2.0 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound was obtained as white solid (21.2 mg, 29%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.28 (m, 2H), 8.98 (br s, 1H), 8.64 (s, 1H), 8.14 (s, 1H), 8.04 (s, 1H), 7.83 (m, 2H), 7.67 (br s, 1H), 5.24 (m, 1H), 2.53 (d, 3H, *J* = 4.8 Hz), 2.15-1.95 (m, 4H), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 452 $(M+H)^+$. HPLC purity: > 98%, RT: 1.16 min.

<u>*N*-(1-(5-(1*H*-Benzo[d][1,2,3]triazol-5-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (20)</u>

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (30 mg, 0.065 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-benzo[d][1,2,3]triazole (31.9 mg, 0.13 mmol), $PdCl_2$(dppf) (9.5 mg, 0.013 mmol) and potassium carbonate (27 mg, 0.195 mmol) in 1/1 degassed solution of DME/H_2O (1.2 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to

room temperature. The title compound **20** was obtained as white solid (1.4 mg, 4%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.27 (s, 1H), 9.17 (br s, 1H), 8.62 (s, 1H), 8.38 (d, 1H, *J* = 9.5 Hz), 8.16 (br s, 1H), 8.15-8.12 (m, 1H), 8.05 (d, 1H, *J* = 8.7 Hz), 7.86 (d, 1H, *J* = 8.5 Hz), 7.67 (br m, 1H), 5.25 (dd, 1H, *J* = 11.6, 8.9 Hz), 2.60-2.50 (m, 3H), 2.08-2.05 (m, 2H), 2.05-2.01 (m, 2H), 1.54-1.57 (m, 2H), 1.46-1.36 (m, 2H), 1.34-1.28 (m, 2H). LCMS (ES^+) m/z 453 $(M+H)^+$. HPLC purity: > 98%, RT: 0.75 min.

_N_-(7-(methylamino)-7-oxo-1-(5-(quinolin-7-yl)-1_H_-imidazol-2-yl)heptyl)thiazole-5-carboxamide (21)

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (25 mg, 0.054 mmol), quinolin-7-ylboronic acid (18.8 mg, 0.108 mmol), $PdCl_2$(dppf) (7.9 mg, 0.0108 mmol) and potassium carbonate (22.5 mg, 0.163 mmol) in 1/1 degassed solution of DME/H_2O (1.0 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound **21** was obtained as pale yellow solid (6.2 mg, 25%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.25 (s, 1H), 9.23 (br s, 1H), 9.08 (dd, 1H, *J* = 4.7, 1.6 Hz), 8.71 (d, 1H, *J* = 8.3 Hz), 8.62 (s, 1H), 8.51 (s, 1H), 8.25 (d, 1H, *J* = 8.7 Hz), 8.26 (s, 1H), 8.12 (br d, 1H, *J* = 6.0 Hz), 7.77 (dd, 1H, *J* = 8.2, 4.7 Hz), 7.68 (br m, 1H), 5.25 (dd, 1H, *J* = 9.0, 6.1 Hz), 2.60-2.50 (m, 3H), 2.08-2.05 (m, 2H), 2.05-2.02 (m, 2H), 1.54-1.47 (m, 2H), 1.47-1.38 (m, 2H), 1.35-1.28 (m, 2H). LCMS (ES^+) m/z 463 $(M+H)^+$. HPLC purity: > 95%, RT: 0.80 min.

_N_-(1-(5-(Isoquinolin-7-yl)-1_H_-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide mono _L_-tartrate salt (22)

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (80 mg, 0.173 mmol), isoquinolin-7-ylboronic acid (60 mg, 0.3468 mmol), $PdCl_2$(dppf) (25.4 mg, 0.035 mmol) and potassium carbonate (72 mg, 0.520 mmol) in 1/1 degassed solution of DME/H_2O (3.2 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound **22** was obtained as TFA salt (54 mg, 45%). This compound was partitioned between DCM and sat. aq. $NaHCO_3$. The organic phase was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting white solid (35.8 mg, 0.077394 mmol) was dissolved in MeCN/H_2O (2:3) and treated with L-tartaric acid (10.45 mg, 0.069654 mmol). The resulting solution was lyophilized to obtain the title compound as mono tartrate salt (45 mg, 95% from TFA salt). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 12.15 (br s, 1H), 9.29 (s, 1H), 9.24 (s, 1H), 9.06 (d, 1H, *J* = 8.4 Hz), 8.62 (s, 1H), 8.42 (d, 2H, *J* =

5.6 Hz), 8.19 (d, 1H, *J* = 8.4 Hz), 7.93 (d, 1H, *J* = 8.8 Hz), 7.76 (d, 1H, *J* = 5.6 Hz), 7.74 (m, 1H), 7.65 (m, 1H), 5.14 (m, 1H), 4.29 (s, 2H, CH tartrate), 2.53 (d, 3H, *J* = 4.4 Hz), 2.09 (m, 1H), 2.04 (t, 2H, *J* = 7.4 Hz), 1.94 (m, 1H), 1.51 (m, 2H), 1.35 (m, 4H). LCMS (ES^+) m/z 463 $(M+H)^+$. HPLC purity: > 99%, RT: 0.66 min.

_N_-(7-(Methylamino)-7-oxo-1-(5-(3-(trifluoromethyl)isoquinolin-7-yl)-1_H_-imidazol-2-yl)heptyl)thiazole-5-carboxamide (23)

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (30 mg, 0.065 mmol), 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)isoquinoline **51** (37.1 mg, 0.13 mmol), $PdCl_2$(dppf) (9.5 mg, 0.013 mmol) and potassium carbonate (27 mg, 0.195 mmol) in 1/1 degassed solution of DME/H_2O (1.2 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound was obtained as pale yellow solid (22.6 mg, 46%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.49 (s, 1H), 9.28 (s, 1H), 9.20 (d, 1H, *J* = 6.4 Hz), 8.64 (s, 2H), 8.47 (s, 1H), 8.35 (m, 1H), 8.26 (m, 1H), 8.10 (br s, 1H), 7.67 (m, 1H), 5.22 (m, 1H), 2.53 (d, 3H, *J* = 4.8 Hz), 2.20-1.95 (m, 2H), 2.05 (t, 2H, *J* = 7.4 Hz), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 531 $(M+H)^+$. HPLC purity: > 99%, RT: 1.33 min.

_N_-(1-(5-(3-Methoxyisoquinolin-7-yl)-1_H_-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (24)

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (30 mg, 0.065 mmol), 3-methoxy-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline **52** (37.1 mg, 0.13 mmol), $PdCl_2$(dppf) (9.5 mg, 0.013 mmol) and potassium carbonate (27 mg, 0.195 mmol) in 1/1 degassed solution of DME/H_2O (1.2 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound was obtained as pale yellow solid (26 mg, 55%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.29 (s, 1H), 9.27 (d, 1H, *J* = 6.4 Hz), 9.11 (s, 1H), 8.64 (s, 2H), 8.44 (s, 1H), 8.10 (br s, 1H), 8.05 (m, 1H), 7.95 (m, 1H), 7.69 (m, 1H), 7.24 (s, 1H), 5.25 (m, 1H), 3.98 (s, 3H), 2.53 (d, 3H, *J* = 4.4 Hz), 2.20-1.95 (m, 2H), 2.05 (t, 2H, *J* = 7.4 Hz), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 493 $(M+H)^+$. HPLC purity: > 95%, RT: 1.00 min.

***N*-(1-(5-(1-methoxyisoquinolin-7-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (25)**

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (30 mg, 0.065 mmol), 1-methoxy-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline **53** (37.1 mg, 0.13 mmol), $PdCl_2$(dppf) (9.5 mg, 0.013 mmol) and potassium carbonate (27 mg, 0.195 mmol) in 1/1 degassed solution of DME/H_2O (1.2 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound was obtained as pale yellow solid (24 mg, 52%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.29 (s, 1H), 9.25 (d, 1H, *J* = 6.4 Hz), 8.64 (s, 1H), 8.61 (s, 1H), 8.16 (d , 2H, *J* = 8.8 Hz), 8.07-8.01 (m, 2H), 7.67 (m, 1H), 7.43 (d, 1H, *J* = 5.6 Hz), 5.25 (m, 1H), 4.11 (s, 3H), 2.54 (d, 3H, *J* = 4.4 Hz), 2.09 (m, 2H), 2.05 (t, 2H, *J* = 7.2 Hz), 1.54-1.24 (m, 6H). LCMS (ES^+) m/z 493 $(M+H)^+$. HPLC purity: > 95%, RT: 1.05 min.

***N*-(1-(5-([1,1'-Biphenyl]-4-yl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (26)**

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (25 mg, 0.0542 mmol), [1,1'-biphenyl]-4-ylboronic acid (21.5 mg, 0.1084 mmol), $PdCl_2$(dppf) (7.9 mg, 0.01084 mmol) and potassium carbonate (22.5 mg, 0.1626 mmol) in 1/1 degassed solution of DME/H_2O (1.0 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound was obtained as pale yellow solid (5.2 mg, 16%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.26 (s, 1H), 9.23 (br s, 1H), 8.61 (s, 1H), 8.08 (s, 1H), 7.87 (d, 1H, *J* = 8.4 Hz), 7.83 (d, 1H, *J* = 6.1 Hz), 7.77 (d, 2H, *J* = 7.7 Hz), 7.68 (br m, 1H), 7.50 (t, 2H, *J* = 7.6 Hz), 7.41 (t, 1H, *J* = 7.4 Hz), 5.24 (t, 2H, *J* = 7.6 Hz), 2.60-2.50 (m, 3H), 2.10-2.05 (m, 1H), 2.05-2.02 (m, 2H), 1.53-1.46 (m, 2H), 1.45-1.38 (m, 2H), 1.32-1.25 (m, 2H). LCMS (ES^+) m/z 488 $(M+H)^+$. HPLC purity: > 99%, RT: 1.41 min.

***N*-(1-(5-(4-(1*H*-Pyrazol-1-yl)phenyl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (28)**

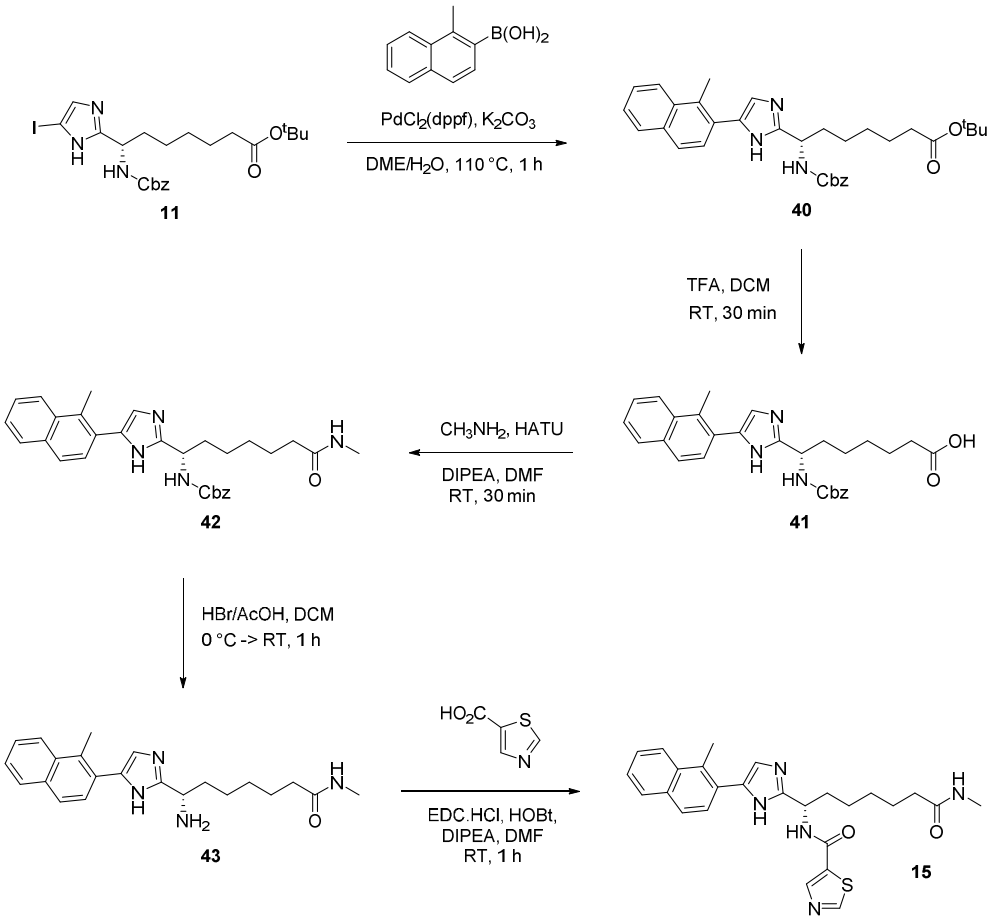
Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (20 mg, 0.043 mmol), (4-(1*H*-pyrazol-1-yl)phenyl)boronic acid (16.3 mg, 0.086 mmol), $PdCl_2$(dppf) (6.1 mg, 0.0086 mmol) and potassium carbonate (18 mg, 0.13 mmol) in 1/1 degassed solution of DME/H_2O (1.0 mL). The title compound was obtained as white solid (12.6 mg, 49%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.29 (s,

1H), 9.26 (d, 1H, *J* = 6.0 Hz), 8.64 (s, 1H), 8.59 (d, 1H, *J* = 2.4 Hz), 8.06 (br s, 1H), 8.00 (m, 2H), 7.92 (m, 2H), 7.79 (m, 1H, *J* = 0.8 Hz), 7.67 (m, 1H), 6.59 (m, 1H), 5.24 (m, 1H), 2.53 (d, 3H, *J* = 4.4 Hz), 2.15-1.95 (m, 2H), 2.05 (t, 2H, *J* = 7.4 Hz), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 478 $(M+H)^+$. HPLC purity: > 99%, RT: 0.97 min.

<u>*N*-(1-(5-(4-(1*H*-Pyrazol-5-yl)phenyl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide mono *L*-tartrate salt (29)</u>

Prepared according to general Suzuki coupling procedure using (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **13** (60 mg, 0.13 mmol), (4-(1*H*-pyrazol-5-yl)phenyl)boronic acid (48.4 mg, 0.26 mmol), $PdCl_2$(dppf) (18.2 mg, 0.026 mmol) and potassium carbonate (53.9 mg, 0.39 mmol) in 1/1 degassed solution of DME/H_2O (2.6 mL). The title compound was obtained as white solid (48 mg, 62%). The product was obtained as TFA salt which was partitioned between DCM and sat. aq. $NaHCO_3$. The organic phase was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting white solid (34 mg, 0.071 mmol) was dissolved in MeCN/H_2O (1:1) (10 mL) and treated with L-tartaric acid (10.7 mg, 0.071 mmol). The resulting solution was lyophilized to obtain the title compound as mono tartrate salt (43.5 mg, 85% from TFA salt). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 13.50-12.50 (br m, 4H), 9.23 (s, 1H), 9.03 (d, 1H, *J* = 8.0 Hz), 8.61 (s, 1H), 7.85-7.45 (m, 7H), 6.69 (d, 1H *J* = 2.0 Hz), 5.11 (m, 1H), 4.30 (s, 2H, CH tartrate), 2.53 (d, *J* = 4.4 Hz), 3H), 2.15-2.00 (m, 3H), 2.00-1.80 (m, 1H), 1.55-1.42 (m, 2H), 1.42-1.20 (m, 4H). LCMS (ES^+) 478 $(M+H)^+$. HPLC purity: > 99%, RT: 0.86 min. ^{13}C-NMR (DMSO-d_6, 150 MHz) δ 159.0, 148.8, 148.0, 145.2, 133.6, 132.7, 132.6, 126.4, 126.3, 115.3, 103.0, 47.5, 35.7, 28.6, 25.5, 25.4. HRMS calcd for $[C_{24}H_{28}N_7O_2S + H]^+$: 478.2020, found: 478.2018.

Scheme 3: Synthesis of compound 15



(*S*)-*tert*-Butyl 7-(((benzyloxy)carbonyl)amino)-7-(5-(1-methylnaphthalen-2-yl)-1*H*-imidazol-2-yl)heptanoate (40)

Prepared according to general Suzuki coupling procedure using (*S*)-tert-butyl 7-(((benzyloxy)carbonyl)amino)-7-(5-iodo-1H-imidazol-2-yl)heptanoate **11** (50 mg, 0.095 mmol), (1-methylnaphthalen-2-yl)boronic acid (34.9 mg, 0.190 mmol), $PdCl_2$(dppf) (13.3 mg, 0.019 mmol) and potassium carbonate (39.4 mg, 0.285 mmol) in 1/1 degassed solution of DME/H_2O (1.9 mL). Reaction mixtures were diluted with EtOAc and filtered on a pad of Solka Floc. Filtrate was washed with sat. aq. $NaHCO_3$, brine, dried (Na_2SO_4), filtered and concentrated to give a crude product which was purified by column chromatography on SiO_2 (eluent: Petroleum ether/EtOAc from 95/5 to 1/1 v/v). The title compound was obtained as pale yellow solid (44.9 mg, 43%). LCMS (ES^+) m/z 542 $(M+H)^+$. HPLC purity: > 90%, RT: 1.87 min.

(*S*)-7-(((Benzyloxy)carbonyl)amino)-7-(5-(1-methylnaphthalen-2-yl)-1*H*-imidazol-2-yl)heptanoic acid (41)

Prepared as described for compound **32** using **40a** (44.9 mg, 0.0829 mmol), DCM (0.5 mL) and TFA (0.5 mL). The title product was obtained after 30 min stirring at room temperature as pale brown sticky solid (100.2 mg, quantitative yield). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.93 (d, 1H, *J* = 6.8 Hz), 7.58 (s, 1H), 7.44-7.28 (m, 5H), 5.04 (dd, 2H, *J* = 12.6, 32.2 Hz), 4.72 (m, 1H), 2.18 (t, 2H, *J* = 7.2 Hz), 1.90-1.70 (m, 2H), 1.53-1.40 (m, 2H), 1.35-1.15 (m, 4H). LCMS (ES^+) m/z 486 $(M+H)^+$. HPLC purity: > 90% (crude), RT: 1.55 min.

Benzyl (7-(methylamino)-1-(5-(1-methylnaphthalen-2-yl)-1*H*-imidazol-2-yl)-7-oxoheptyl)carbamate (42)

Prepared as described for compound **33** using **41a** (40.2 mg, 0.0829 mmol), HATU (63 mg, 0.1658 mmol), DIPEA (0.043 mL, 0.2487 mmol), methylamine (2.0 M solution in THF, 0.21 mL, 0.4145 mmol) and DMF (1 mL). The reaction mixture was stirred at room temperature for 1 h, diluted with EtOAc, washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude was purified by column chromatography (eluent: Petroleum ether/EtOAc from 9/1 to 0/10 v/v and EtOAc/MeOH 95/5 v/v) to get the title compound as a pale yellow solid (32 mg, 77%). LCMS (ES^+) m/z 499 $(M+H)^+$. HPLC purity: > 90%, RT: 1.51 min.

7-Amino-*N*-methyl-7-(5-(1-methylnaphthalen-2-yl)-1*H*-imidazol-2-yl)heptanamide (43)

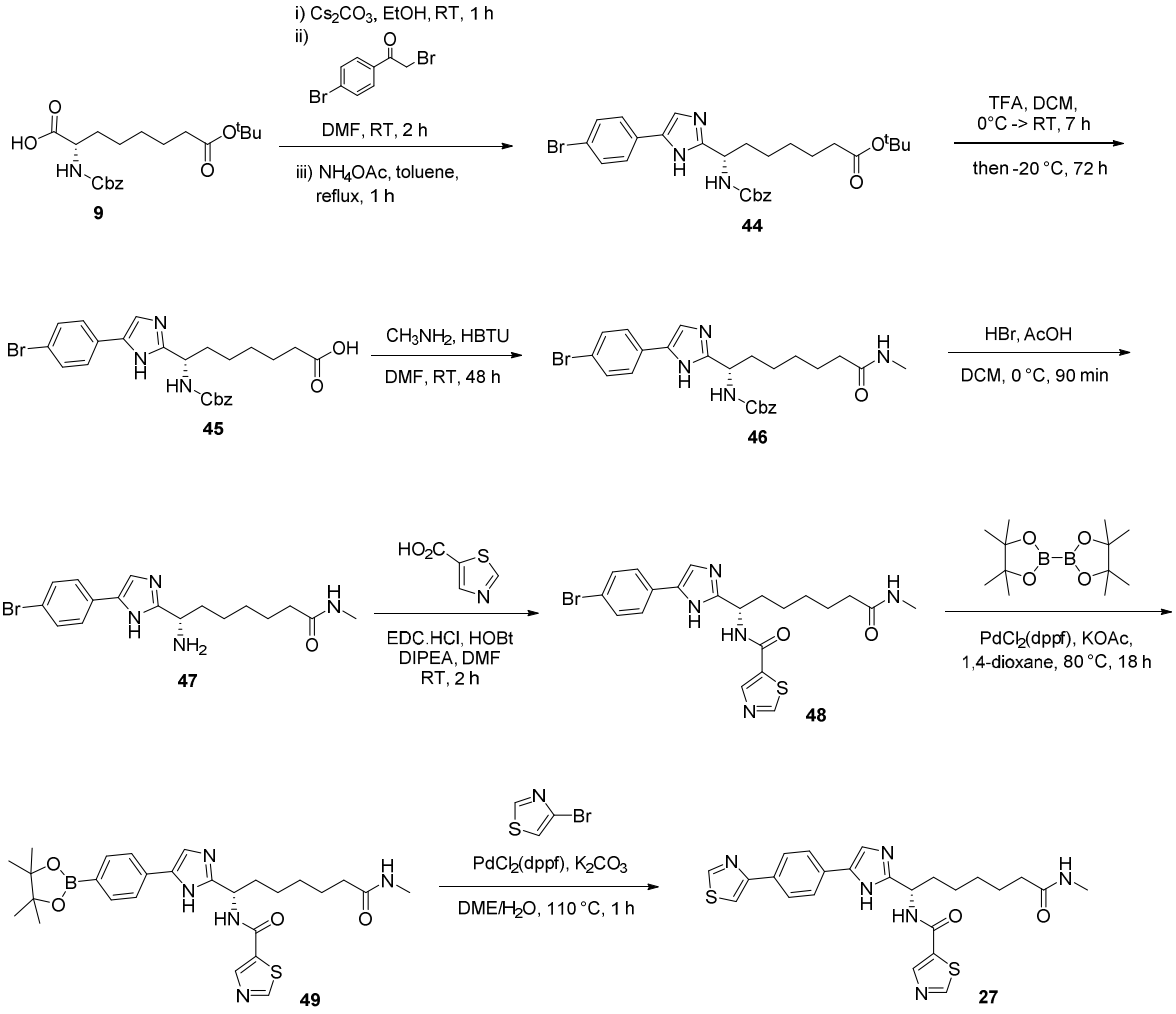
Prepared as described for compound **39** using **42a** (32 mg, 0.064 mmol), DCM (0.4 mL) and HBr in AcOH (0.2 mL). The title compound was obtained after h at 0°C and 1 h at room temperature as a glassy oil (21 mg, 90%). LCMS (ES^+) m/z 365 $(M+H)^+$. HPLC purity: > 90%, RT: 1.11 min.

***N*-(7-(Methylamino)-1-(5-(1-methylnaphthalen-2-yl)-1*H*-imidazol-2-yl)-7-oxoheptyl)thiazole-5-carboxamide (15)**

Prepared according to general amide coupling procedure using **43a** (21 mg, 0.058 mmol), thiazole-5-carboxylic acid (9.7 mg, 0.075 mmol), HOBt (10.4 mg, 0.075 mmol) and EDC·HCl (14.4 mg, 0.075 mmol) in DMF (1.0 mL). The title compound was obtained as pale yellow solid (13 mg, 47%). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.25 (s, 1H), 9.28-9.22 (br s, 1H), 8.60 (s, 1H), 8.16 (d, 1H, *J* = 8.3 Hz), 7.91 (d, 1H, *J* = 8.4 Hz), 7.86 (d, 1H, *J* = 2.3 Hz), 7.68 (t, 1H, *J* = 7.6 Hz), 7.67 (br s, 1H), 7.63 (t, 1H, *J* = 7.6 Hz), 7.56 (d, 1H, *J* = 7.2 Hz), 7.51 (d, 1H, *J* = 7.3 Hz), 5.29 (dd, 1H, *J* = 16.0, 7.3 Hz), 2.72 (s, 3H), 2.60-2.50 (m, 3H), 2.10-2.06 (m, 2H), 2.08-2.03 (m, 2H), 1.54-1.48 (m,

2H), 1.48-1.40 (m, 2H), 1.37-1.27 (m, 2H). LCMS (ES^+) m/z 476 $(M+H)^+$. HPLC purity: > 99%, RT: 1.17 min.

Scheme 4: Synthesis of compound 27



(*S*)-*tert*-Butyl 7-(((benzyloxy)carbonyl)amino)-7-(5-(4-bromophenyl)-1*H*-imidazol-2-yl)heptanoate (44)

To a stirred solution of (*S*)-2-(((benzyloxy)carbonyl)amino)-8-(*tert*-butoxy)-8-oxooctanoic acid **9** (prepared as described in WO2006/061638) (4.82 g, 12.7 mmol) in EtOH (25 mL) Cs_2CO_3 (2.07 g, 6.35 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. Solvent was evaporated under reduced pressure and the resulting solid was dissolved in DMF (25 mL) and treated with 2-bromo-1-(4-bromophenyl)ethanone (3.53 g, 12.7 mmol) for 2 h at room temperature. The reaction mixture was then diluted with Toluene and concentrated under reduced pressure to

afford an orange oily residue. A solution of this oil and ammonium acetate (1.96 g, 25.4 mmol) in toluene (100 mL) was refluxed under Dean-Stark conditions for 1 h. After cooling to room temperature the mixture was diluted with EtOAc. The organic phase was washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure to give a crude that was purified by column chromatography (eluent: petroleum ether/EtOAc from 9:1 to 1:1) to give the title compound as a yellow powder (2.47 g, 35%). LCMS (ES^+) m/z 556, 558 $(M+H)^+$. HPLC purity: > 90%, RT: 1.81 min.

(*S*)-7-(((Benzyloxy)carbonyl)amino)-7-(5-(4-bromophenyl)-1*H*-imidazol-2-yl)heptanoic acid (45)

Prepared as described for compound **11** using **44** (2.41 g, 4.3379 mmol), DCM (40 mL) and TFA (4 mL). The title product was obtained after 7 h at room temperature and 72 h at -20 °C as pale brown sticky solid (2.66 g, quantitative yield). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.93 (d, 1H, J = 6.8 Hz), 7.58 (s, 1H), 7.44-7.28 (m, 5H), 5.04 (dd, 2H, J = 12.6, 32.2 Hz), 4.72 (m, 1H), 2.18 (t, 2H, J = 7.2 Hz), 1.90-1.70 (m, 2H), 1.53-1.40 (m, 2H), 1.35-1.15 (m, 4H). LCMS (ES^+) m/z 500, 502 $(M+H)^+$. HPLC purity: > 90%, RT: 1.41 min.

(*S*)-Benzyl (1-(5-(4-bromophenyl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate (46)

To a solution of **45** (2.66 g, 4.3 mmol) in DMF (17 mL) were added methylamine (2.0 M solution in THF, 21.5 mL, 43 mmol) followed by HBTU (2.44 g, 6.45 mmol) and the resulting solution was stirred for 18 h at room temperature. Then HBTU and methylamine were added until completion of reaction was observed (48 h). The reaction mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude was purified by column chromatography (eluent: DCM/MeOH from 100:0 to 9:1) to get the title compound as a pale yellow solid (1.61 g, 73%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.59 (m, 2H), 7.48 (d, 2H, J = 8.4 Hz), 7.36 (m, 4H), 7.22 (s, 1H), 5.65-5.55 (m, 2H), 5.13 (dd, 2H, J = 12.4, 16.8 Hz), 4.75 (m, 1H), 2.81 (d, 3H, J = 4.8 Hz), 2.25-2.05 (m, 3H), 1.95 (m, 1H), 1.80-1.50 (m overlapped with H_2O, 2H), 1.50-1.30 (m, 4H). LCMS (ES^+) m/z 513, 515 $(M+H)^+$. HPLC purity: > 95%, RT: 1.38 min.

(*S*)-7-Amino-7-(5-(4-bromophenyl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide (47)

Prepared as described for compound **39** using **46** (800 mg, 1.5 mmol), DCM (4 mL) and HBr in AcOH (8 mL). The title compound was obtained after 1 h 30 min at 0 °C as an orange oil (569 mg, quantitative yield). LCMS (ES^+) m/z 379, 381 $(M+H)^+$. HPLC purity: > 90%, RT: 1.02 min.

(*S*)-*N*-(1-(5-(4-Bromophenyl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (48)

Prepared according to general amide coupling procedure using **47** (569 mg, 1.5 mmol), thiazole-5-carboxylic acid (290 mg, 2.25 mmol), HOBt (304 mg, 2.25 mmol), EDC·HCl (431 mg, 2.25 mmol) and DIPEA (788 µL, 4.5 mmol) in DMF (7 mL). The reaction mixture was stirred at room temperature for 2 h, then diluted with EtOAc, washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude was purified by column chromatography (eluent: DCM/MeOH from 100:0 to 95:5) to get the title compound as a pale yellow (291 mg, 40%). LCMS (ES^+) m/z 490, 492 $(M+H)^+$. HPLC purity: > 90%, RT: 1.09 min.

(*S*)-*N*-(7-(Methylamino)-7-oxo-1-(5-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1*H*-imidazol-2-yl)heptyl)thiazole-5-carboxamide (49)

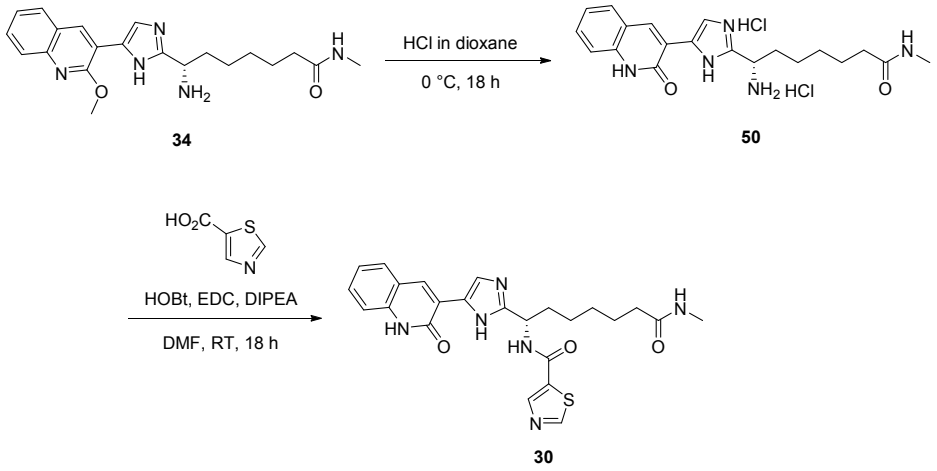
A degassed microwave vial was charged with **48** (50.7 mg, 0.103 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (52.5 mg, 0.206 mmol), $PdCl_2$(dppf) (7.5 mg, 0.010 mmol) and potassium acetate (25.3 mg, 0.257 mmol). Pre-degassed 1,4-dioxane (1.0 mL) was added and the suspension was degassed for further 10 minutes and then heated at 80 °C for 18 h. After cooling, reaction mixture was diluted with EtOAc and filtered on a pad of Solka Floc. Filtrate was washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude product was used in the next step without further purification. LCMS (ES^+) m/z 538 $(M+H)^+$.

(*S*)-*N*-(7-(Methylamino)-7-oxo-1-(5-(4-(thiazol-4-yl)phenyl)-1*H*-imidazol-2-yl)heptyl)thiazole-5-carboxamide (27)

Prepared according to general Suzuki coupling procedure using **49** (27.7 mg, 0.052 mmol), 4-bromothiazole (4.6 µL, 0.052 mmol), $PdCl_2$(dppf) (7.5 mg, 0.010 mmol) and potassium carbonate (21.4 mg, 0.155 mmol) in 1/1 degassed solution of DME/H_2O (1.0 mL). Reaction mixture was heated at 110 °C for 1 h, then cooled to room temperature. The title compound **27** was obtained as white solid (6.3 mg, 20%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.30 (s, 1H), 9.28 (br s, 1H), 9.24 (s, 1H), 8.64 (s, 1H), 8.30 (s, 1H), 8.15 (d, 2H, J = 8.4 Hz), 8.11 (br s, 1H), 7.89 (d, 2H, J = 8.4 Hz),

7.68 (m, 1H), 5.25 (m, 1H), 2.53 (d, 3H, J = 4.4 Hz), 2.15- 1.95 (m, 4H), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 495 $(M+H)^+$. HPLC purity: > 99%, RT: 1.05 min.

Scheme 5: Synthesis of compound 30



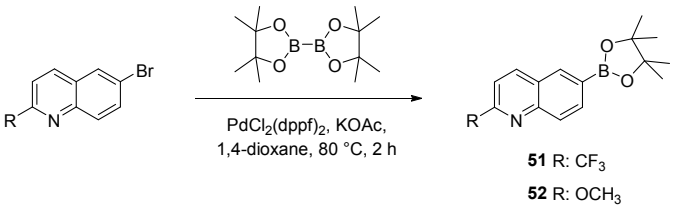
(*S*)-7-Amino-*N*-methyl-7-(5-(2-oxo-1,2-dihydroquinolin-3-yl)-1*H*-imidazol-2-yl)heptanamide trihydrochloride (50)

Compound **34** (58 mg, 0.152 mmol) was treated with 4M HCl/1,4-dioxane solution (0.6 mL) at 0 °C then the reaction mixture was stirred at room temperature for 4h. Volatiles were evaporated under reduced pressure affording the title compound as pale yellow solid (72.5 mg, quantitative yield). LCMS (ES^+) m/z 368 $(M+H)^+$.

(*S*)-*N*-(7-(Methylamino)-7-oxo-1-(5-(2-oxo-1,2-dihydroquinolin-3-yl)-1*H*-imidazol-2-yl)heptyl)thiazole-5-carboxamide (30)

Prepared according to general amide coupling procedure using **50** (58 mg, 0.112 mmol), thiazole-5-carboxylic acid (23.6 mg, 0.183 mmol), HOBt (24.7 mg, 0.183 mmol), EDC·HCl (35 mg, 0.183 mmol) and DIPEA (95 µL, 0.547 mmol) in DMF (1.0 mL). The title compound was obtained as TFA salt (28 mg, 48%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 12.23 (br s, 1H), 9.28 (s, 1H), 9.18 (br s, 1H), 8.63 (s, 1H), 8.50 (s, 1H), 8.10 (br s, 1H), 7.75 (d, 1H, J = 8.0 Hz), 7.67 (m, 1H), 7.56 (m, 1H), 7.39 (d, 1H, J = 8.4 Hz), 7.26 (t, 1H, J = 7.4 Hz), 5.26 (m, 1H), 2.54 (d, 3H, J = 4.8 Hz), 2.15-1.95 (m, 4H), 1.55-1.20 (m, 6H). LCMS (ES^+) m/z 479 $(M+H)^+$. HPLC purity: > 99%, RT: 0.90 min.

Experimental details for the synthesis of boronic ester intermediates 51, 52 and 53

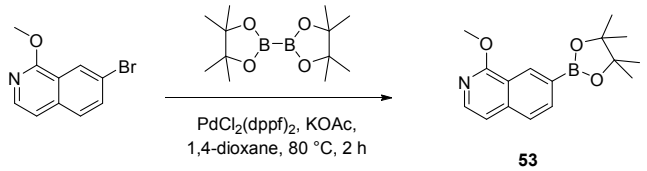


7-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl)isoquinoline (51)

Prepared as described for compound **49** using 6-bromo-2-(trifluoromethyl)quinoline (50 mg, 0.1811 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (92 mg, 0.362 mmol), $PdCl_2$(dppf) (13.2 mg, 0.018 mmol) and potassium acetate (44.4 mg, 0.453 mmol) in 1,4-dioxane (1.8 mL) and heated at 80 °C for 2 h. After cooling, the reaction mixture was diluted with EtOAc, washed with sat. aq. $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (eluent: petroleum ether/EtOAc from 95:5 to 50:50) to get the title compound as a pale yellow solid (58.5 mg, quantitative yield). LCMS (ES^+) m/z 324 $(M+H)^+$.

2-Methoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (52)

Prepared as described for compound **49** using 6-bromo-2-methoxyquinoline (50 mg, 0.21 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (106.7 mg, 0.42 mmol), $PdCl_2$(dppf) (15.4 mg, 0.021 mmol) and potassium acetate (51.5 mg, 0.525 mmol) in 1,4-dioxane (2.1 mL) and heated at 80 °C for 2 h. The same work up and purification of compound **51** were performed affording the title compound as a pale yellow solid (50 mg, 83%). LCMS (ES^+) m/z 286 $(M+H)^+$.



1-Methoxy-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (53)

Prepared as described for compound **49** using 7-bromo-1-methoxyisoquinoline (200 mg, 0.84 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (427 mg, 1.68 mmol), $PdCl_2$(dppf) (62 mg, 0.084 mmol) and potassium acetate (207 mg, 2.1 mmol) in 1,4-dioxane (4.5 mL) and

heated at 80 °C for 2 h. The same work up and purification of compound **51** were performed affording the title compound as a pale yellow solid (200 mg, 83%). LCMS (ES^+) m/z 286 $(M+H)^+$.

***Pf*growth, HeLa Class I HDAC and *h*HDAC1 assays procedures**

Compound preparation

Compounds, to be transferred both to biochemical and cell based assay, are prepared from 10 mM DMSO stock solutions. Mother solutions are serially diluted and transferred to assay plates by an acoustic droplet ejection device (ATS-100, EDC biosystems, USA).

Parasite Culture

P. falciparum 3D7 strain parasites were cultured in type A/0+ human erythrocytes at 5% hematocrit and 0.5-10% parasitemia in RPMI 1640 (Life Technologies) supplemented with 5% Albumax (Life Technologies) and 0.36 mM hypoxanthine by using established methods (Trager and Jensen 1976). Parasite were cultured at 37 °C in modified atmosphere (3% O_2, 4% CO_2 and N_2).

*Pf*growth assay

Parasite proliferation assays were performed in 384-well plates (Thermo, 4332, USA) with a starting parasitemia of 0.25% at a hematocrit of 2% by quantification of parasite lactate dehydrogenase (pLDH). Serial dilution of compounds or DMSO as control were pre dispensed on plate as previously described. Assay plates are incubated for 72 hours at 37 °C in 5% CO_2, 5% O_2, and 95% N_2 containing atmosphere. After the incubation period, plates are frozen at – 80 °C for two hours and then thawed at room temperature for 2 hours. Then, 50 µL of the developer solution (70 mM TrisHCl pH 8.0, 0.5% Tween-20, 100 mM Lithium L-Lactate, 125 µM nitro blue tetrazolium, 2 U/ml diaphorase and 100 µM 3-Acetylpyridine Adenine Dinucleotide) are added to each well and let to react for 10 minutes. Absorbance is then read at 650 nm by a suitable spectrophotometer.

HeLa Class I HDAC inhibition assay

The buffer used in the assay is TBS + 0.25 mM $MgCl_2$ + 0.02% BSA. HeLa cells are plated in DMEM without phenol red (Thermo, 11880, USA + 10% FBS + 1X PenStrep + 1X Gln) to a density of 10000 cells per well in a 384 well plate (Thermo, 4334-11, USA) and let recover for four hours at 37 °C, 5% CO_2 in a humidified atmosphere. After the recovery, compounds are transferred to assay plates as per compound preparation method. Then, 5 µL of assay buffer diluted substrate (Enzo, BML-KI104, USA) are added to each well to a final concentration of 400 µM. The reaction

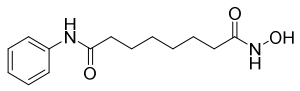
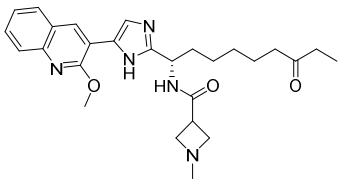
is incubated four hours 37 °C, 5% CO_2 in a humidified atmosphere. To develop the reaction signal, 15 µL of 27x assay buffer diluted developer solution (Enzo, BML-KI105, USA) are transferred to each well with the addition of 3% NP40 and 6 µM final concentration dacinostat (Selleckchem, S1095, USA) to stop the reaction. After 10 minutes incubation at room temperature the reaction signal is red at 360 nm excitation, 460 nm emission on a suitable spectrophotometer.

Human HDAC1 activity assay

The buffer used in the assay is TBS + 1 mM $MgCl_2$ + 0.1% BSA. Human HDAC1 (Enzo, BML-SE456, USA) is diluted to 1 nM in assay buffer. 15 µL of the diluted enzyme mix are transferred to each well of compound containing microplates (Thermo, 4316, USA). After 10 minutes incubation at room temperature, 5 µL of assay buffer diluted substrate (Enzo, BML-KI104, USA) are added to each well to a final concentration of 80 µM. The reaction is incubated for one hour at room temperature. To develop the reaction signal, 15 µL of 600x assay buffer diluted developer solution (Enzo, BML-KI105, USA) are transferred to each well with the addition of 3 µM final concentration dacinostat (Selleckchem, S1095, USA) to stop the reaction. After 10 minutes incubation at room temperature the reaction signal is red at 360 nm excitation, 460 nm emission on a suitable spectrophotometer.

Biological data for compound 1 (vorinostat, SAHA) and 3 (standard reference)

Supplementary Table 1.

compound	Structure	*Pf*growth EC_{50} [b]	HeLa class I HDAC IC_{50} [b]	*h*HDAC1 IC_{50} [b]	SI [c]
1		0.21 ± 0.02	0.61 ± 0.01	0.03 ± 0.01	3
3		0.21 ± 0.07	0.07 ± 0.02	0.002 ± 0.001	0.3

[a] IC_{50} and EC_{50} values in µM. [b] IC_{50} and EC_{50} values are the average of at least three individual measurements ± SD. [c] SI: Selectivity Index (HeLa class I HDAC(µM)/*Pf*growth(µM)).

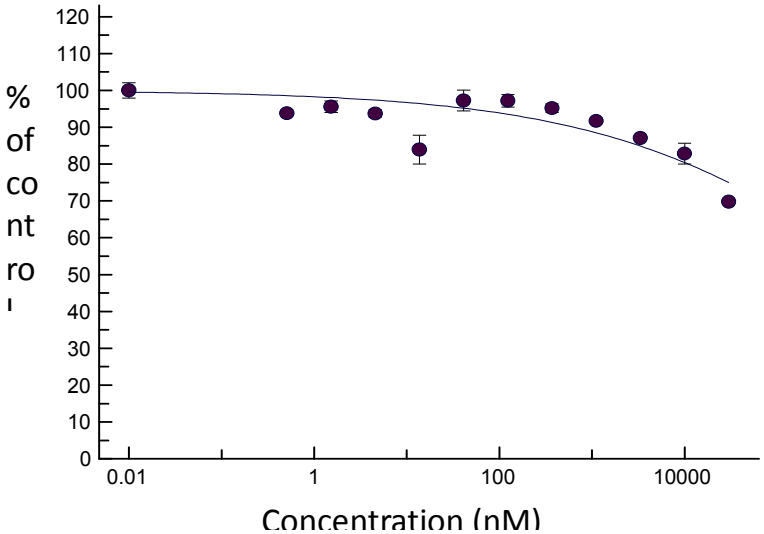
HeLa and HUVEC cytotoxicity assays procedure

HeLa cells (DMEM without phenol red, Thermo, 11880, USA + 10% FBS + 1X PenStrep + 1X Gln) or HUVEC cells (EBM-2, Lonza, CC3156 + EGM-2 SingleQuotsTMSupplements and Growth Factors Lonza, CC4176 + 1X PenStrep + 1X Gln) are plated in a 384 well plate (Thermo, 4334-11, USA) to a density of 2000 cells per well and let recover for four hours at 37 °C, 5% CO_2 in a humidified atmosphere. After the recovery, compounds are transferred to assay plates as per compound preparation method. Assay plates are then incubated hours at 37 °C, 5% CO_2 in a humidified atmosphere for 72 hours. Cell viability is measured by the CellTiTer Glo (Promega, G8080, USA) as per manufacturer instruction.

Human Ether-a-go-go-Related Gene (hERG) inhibition assay procedure

The assay employs a membrane fraction containing hERG channel protein (Invitrogen's Predictor™ hERG Fluorescence Polarization Assay) and a high-affinity red fluorescent hERG channel ligand, or "tracer" (Predictor™ hERG Tracer Red), in a fluorescence polarization (FP)-based format. The assay is based on the principle of fluorescence polarization where the redshifted fluorescent tracer is displaced from the hERG channel by compounds that bind to the channel. Lower polarization values correlate to greater displacement of the tracer, and therefore indicate binding to hERG. The decrease of fluorescence polarization was measured using a Tecan ULTRA microplate reader equipped with a fluorescence polarization module. Compound dilutions were made in 100% DMSO starting from 10 mM stock to have 3 mM in DMSO. The highest concentration of 3 mM was used to do a titration curves (10 points, 1:3 dilution). All concentration points were tested in duplicates. The positive control (compound E-4031) was used also starting at 30 µM concentration, as recommended by the manufacturer. The assay was performed in 384-well format. After incubation for 4 hours at room temperature, the fluorescence polarization was measured. The results were expressed as % of inhibition. The IC_{50} value was obtained by fitting the measured fluorescence polarization signal against **29** concentrations using nonlinear regression with XLfit 4.2 (IDBS Ltd, USA).

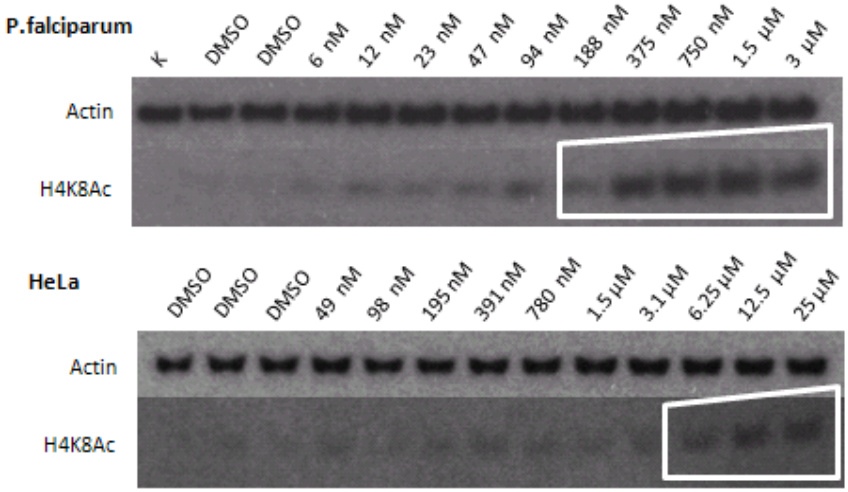
Supplementary Figure 1: Dose-response curve of **29** in the hERG inhibition assay. Data is reported as % of inhibition over vehicle treatment. IC_{50} was greater than 30 μM.



Hyperacetylation of histone H4 by 29 assay procedure

Assessment of parasite histone hyperacetylation were performed in 48-well plates with a starting parasitemia of 5-7% at a hematocrit of 5% by incubating infected erythrocytes for 14-16 hours with serial dilution of compound or DMSO. Treated parasites were obtained from several rounds of RBC lysis in cold PBS containing 0.1% saponin and protease inhibitors. Free parasites were lysed in 2% (wt/vol) SDS by bath sonication. Parasite proteins were separated by SDS/PAGE on NuPAGE 12% Bis-Tris gels (Life Technologies) and transferred to PVDF (Bio-Rad) membrane. Membranes were blocked in TBS-T (25 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween-20, 5% dry no-fat milk) and probed for Anti-acetyl-Histone H4 (Lys8) (07-328, Millipore) diluted 1:2000 or for anti-actine (A2066, Sigma-Aldrich) diluted 1:5000, followed by anti-rabbit-HRP (Bio-Rad 972-4446) diluted 1:5000 in TBS-T, and then revealed by using ECL Western Blotting Detection Reagents (RPN2106, GE Healtcare). Acquisition and densitometry analysis was performed by using ChemiDoc MP Imaging System (Bio-Rad). Assessment of HeLa histone hyperacetylation were performed in 96-well plates with the same procedure described above, with the difference that after the incubation, cells were washed and collected by the addition of 2% (wt/vol) SDS and thereafter sonicated.

Supplementary Figure 2: Hyperacetylation of histone H4 in *P. falciparum* and in HeLa cells after treatment with **29**.



Microsome stability assays procedures

Materials and methods

All reagents were of the highest grade commercially available and were purchased from SIGMA (St. Luis, MO) unless otherwise specified. Wistar Han rat liver microsomes (Cat. No. 452511), CD-1 mouse liver microsomes (Cat. No. 452701) and human liver microsomes (Cat. No. 452161) were purchased from Gentest (BD GENTEST, Woburn, MA). Compounds tested were all in house synthetized. A Hamilton Workstation STARPLUS was used as robotic liquid handler. The instrument was equipped with: 8 standard tips, 96 CO-RE Probe Head, four Thermomixer IKA 260 and two F12 Julabo apparatus, used one as a heater and the other one as a cooling system. Hamilton was controlled via Microlab STAR software version 4.0.0. Plates used were 96-well 2mL deep well PP plates (V-bottomed) from Waters (Waters, Milford, MA) and Matrix (Matrix Chesire,UK).

Preparation of stock solutions

Drug candidates from 10 mM DMSO stock solution are diluted in MeOH:H_2O 1:1 in order to have a 1 µM final concentration in incubation mix, final MeOH concentration is 0.36% (DMSO is 0.01%.); they are contained in 96 MW plate and stored sealed at room temperature until use. Microsomes used in oxidative metabolism are thawed and immediately diluted with KPB according to needing, and maintained at 0 °C until use. Buffers are 0.1 M, potassium phosphate buffer (KPB) at pH 7.4 is used in presence of NADPH cofactors. Cofactors are prepared according to the following protocol: NADPH: a solution in KPB buffer 0.1 M contains: 2.9 mg/ml NADP, 1 mM

final conc. in incubation, 23 mg/ml of G6P, 20 mM final conc. in incubation, 7.6 ul/ml $MgCl_2$ 1M (2 mM in incubation) and G6P-dehydrogenase (final activity: 2U/sample). Buffers, cofactors and microsome stocks are placed into Matrix plates (cod. 1064-05-10) composed of 12 separated wells of 16 mL each. Until use, they are placed in a 4 °C cooled position on the Hamilton station. Final mix volume in incubation is 380 µL: 178 µL are from buffer stocks, 100 uL from microsomes stocks, 2 uL from drug stocks and 100 µL from cofactor stocks. The different components are added in the same order reported here.

Assay protocol

Test compound was incubated at 1 µM concentration with human (20 donor, Cat. No. 452161, Gentest, BD Gentest, Woburn, MA), rat (male Wistar Han, Cat. No. 452511, Gentest, BD Gentest, Woburn, MA) and mouse (male CD-1, Cat. No. 452701, Gentest, BD Gentest, Woburn, MA) liver microsomes at 0.5 mg microsomal protein/mL, at 37 °C for 60 min. The incubation mix was the following: potassium phosphate buffer 0.1 M pH 7.4 (KPB), microsomes (final concentration 0.5 mg microsomal protein/mL), test compound (final concentration 1 µM) and cofactors (NADP, 1 mM final concentration in incubation; G6P, 20 mM final concentration in incubation; $MgCl_2$ 2 mM in incubation; G6P-dehydrogenase final activity: 2 U/sample). The different components were added in the same order reported here. Microsomes incubated in absence of cofactor were also prepared and used as control samples.

Before addition of cofactors the incubation tubes were pre incubated at 37 °C for 5 min. As soon as cofactors were added, an aliquot of the incubation mix was immediately transferred into a quenching plate (t = 0 min) containing one volume of MeCN and internal standard (0.5 µM Dextromethotphan) to stop biotransformation reactions and extract test compound. This sample represents the initial conditions. After fixed elapsed periods (10, 20, 30, 45 and 60 min) others time points were similarly collected. Samples were centrifuged (14,000 rcf for 15 min) and the supernatants were analyzed by liquid chromatography-mass spectrometry.

Sample analysis

Detection of drugs is obtained by LC/MS/MS spectrometry. A Micromass 4 Ultima Platinum Triple Quadrupole and a Waters Acquity UPLC module (Acquity Sample Manager- Acquity Binary Solvent Manager), equipped with a Waters Acquity Sample Organizer Autosampler are used (Waters, Milford, MA), instruments are controlled with Masslinx software. Autosampler's drawers are kept cooled at 8 °C. MS/MS methods are generated automatically with QuanOptimize. Basically a 2 uL of a 5 uM solution of each analyte are injected in the analytical column twice (BEH C18 50

x 2.1 mm, 1.7 micron), the first time to optimize the Q1 condition, scanning the cone voltage from 20V to 60V with step of 10V, the second time in order to scan on Q3 the daughter ions after fragmentation and to select the most intense MRM transition for quantification, collision energies are optimized by scanning from 10V to 40V with steps of 5V. The analytes are eluted during optimization with the following gradient: from 0 to 1 min the percent of B increase from 50% to 99% at 0.3 ml/min, from 1 min to 1.2 min an isocratic gradient is kept at 1 mL/min, from 1.2 to 1.21 min the initial % of B is restored and the system is equilibrated for 0.4 min and the flow is decreased at 0.1 mL/min. Positive Electrospray condition are used, if method fails to detect the analyte, negative ionization mode is explored. During analysis the following gradient is used: flow is 0.8 mL/min, from 0 to 0.44 min the percent of B is linearly increased from 2% to 90% and kept constant for 0.33 min. From 0.79 to 0.82 min then initial conditions are restored and the system is equilibrated for 0.18 min. Injection volume is 2 µL.

Raw data treatment

After analysis, a representative chromatogram is used to rapidly create the methods for the area integration, then all chromatograms are analyzed and area of peaks measured automatically with QuanLynx software. Raw data relative to peaks' area are directly copied and pasted into an Excel datasheet used as template in which cells containing area of peaks are linked to formulas that directly calculates Intrinsic Clearance (Clint). Excel fit software is used to automatically calculate the slope of the logarithmic trend of the residual area percent vs. time, according to the following formula:

$$-\frac{d\left(\ln\frac{s}{s_0}\right)}{dt} = -slope = \frac{V_M}{K_M} = Cl_{\text{int}}$$

Where "s" is the peak area of the compounds at time t and s_0 is the peak area of the compounds at time zero. Calculation is based on the minimization of the sum of the squares of residuals of experimental values from the theoretical plot. Excel fit is also used to calculate standard errors and confidence intervals of the measured intrinsic clearance.

***In vivo* pharmacokinetic studies procedures**

Studies were performed in both rats (Wistar) and mice (NMRI). The study protocols were reviewed and approved by the Institutional Animal Care and Use Committees at Merck Research Laboratories. For studies compounds were dosed as follow:

Compound **6** was dosed intravenously and orally to mice NMRI and rat Wistar, the compound was administered as a bolus at either 2 mg/kg of body weight (i.v.) or 10 mg/kg (p.o.) in mice and at 10 mg/kg (p.o.) in rats. For oral studies, the compound was dosed as a solution in PEG/Water 50/50. For intravenous studies, the compound was dosed as a solution in DMSO/PEG400/Water 20/60/20.

Compound **7** was dosed intravenously and orally to mice, the compound was administered as a bolus at either 2 mg/kg of body weight (i.v.) or 10 mg/kg (p.o.). For oral studies, the compound was dosed as a solution in water tween 9%. For intravenous studies, the compound was dosed as a solution in DMSO/PEG400/Water 20/60/20.

Compound **8** was dosed intravenously and orally to mice and rats, the compound was administered as a bolus at either 2 mg/kg of body weight (i.v.) or 10 mg/kg (p.o.) in mice and at either 1 mg/kg of body weight (i.v.) or 6 mg/kg (p.o.) in rats. For oral studies, the compound was dosed as a solution in water tween 9%. For intravenous studies, the compound was dosed as a solution in DMSO/PEG400/Water 20/60/20.

Compound **29** was dosed intravenously and orally to mice and rats, the compound was administered as a bolus at either 2 mg/kg of body weight (i.v.) or 10 mg/kg (p.o.) in mice and 1 mg/kg of body weight (i.v.) in rats. For oral and intravenous studies, the compound was dosed as a solution in water dextrose 5% with DMSO 90:10.
For all studies, blood samples were collected in EDTA-containing tubes at appropriate times and plasma was separated by centrifugation and stored at −70°C until analysis. Quantitation of 641-642 levels was conducted by high-performance liquid chromatography/mass spectroscopy (LC/MS/MS) following protein precipitation.

Urine analysis for compound 29 after i.v. dosing

Sample Preparation

Urine samples were extracted as follows: 200 µL of MeCN, containing 25 ng/mL of Verapamil as internal standard, was added to 50 µL of rat urine; this sample was vortexed and centrifuged at 4500 rpm for 15 min. The supernatant was transferred into a clean 96 deep-well plate, dried under nitrogen and reconstituted with H_2O/MeCN (90:10). Prepared samples were maintained at 8 °C in autosampler during analysis.

Analytical conditions

Quantitative analysis of test compound was performed by LC-MS/MS using an Agilent 1100 series gradient HPLC pump (Agilent, Palo Alto, CA, USA), a CTC HTS PAL Autosampler (CTC Analytics, Zwingen, Switzerland) coupled to an API400 Qtrap (Applied Biosystems, Foster City, CA, USA) triple quadrupole mass spectrometer equipped with an ESI interface operated in positive-ion mode. Chromatographic separation was performed using a C18 analytical column (XBridge, 3.5 µm, 2.1 x 30 mm ID) at room temperature with an injection volume of 5 µL (loop). The mobile phase, consisting of a solvent A (water with 0.1 % formic acid) and solvent B (MeCN with 0.1 % formic acid), was delivered at a flow rate of 0.25 $mL.min^{-1}$. The LC gradient started from 5 % of B and for 0.5 min remains at 5 %, then from 0.5 to 3.5 min the percent of B is linearly increased to 90 % remaining constant to this ratio for 1.0 min. The gradient decreased to 5 % of B at 4.6 min, remaining constant to this ratio until 6.6 min that was the time of analysis. Detection of compound was performed in the multiple reaction-monitoring (MRM) mode, monitoring the following ion transitions: single charged molecular ion $[M+H]^+$ at m/z 478.3/350.3 and 478.3/251.3. The Internal Standard used (Verapamil) was monitored at m/z 455.1/ 164.0.

Supplementary Table 2: Concentration of compound **29** in urine of wistar rat male after iv dosing (1 mpk)

	Concentrations (ng/ml) of Compound 29 in RAT Male Urine				
		Treatment IV			
Time Hours	Subject 1	Subject 2	Subject 3	Mean	std. Dev.
0 - 2	0.00	3831.63	9012.25	**4281.29**	4522.92
2 - 8	0.00	754.99	4335.19	**1696.73**	2315.95
8 - 24	3919.71	356.95	243.14	**1506.60**	2090.59
Dose Recovery (%)	10				

Comment Note:The carboxilic acid derivative from the amide hydrolysis was detected in urine but quantification was not possible. N-glucuronide and the N-acetyl metabolites were not detected.